Familial Aggregation and Early Expression of Hyperapobetalipoproteinemia

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Family history is an important predictor of coronary risk. However, this relation, in large part, is not explained by the known risk factors such as systemic hypertension or hyperlipidemia. In the present study, plasma lipid, lipoprotein lipid, and plasma low-density lipoprotein (LDL) apoB levels were measured in 66 offspring (myocardial infarction [MI] offspring) of 24 families in which an index parent had premature coronary artery disease and hyperapobetalipoproteinemia. These results were compared to those obtained in 207 control children and young adults. Univariate analysis revealed that plasma LDL apoB and all other lipid and lipoprotein levels except high-density lipoprotein cholesterol were significantly higher in the MI offspring. Multivariate analysis showed plasma LDL apoB and LDL cholesterol best differentiated the MI offspring from control children and young adults. Of the 66 children, 22 had hyperapobetalipoproteinemia, of whom only 7 had clearly abnormal LDL cholesterol or plasma triglyceride levels. Thus, a substantial portion of children born to a parent with premature coronary artery disease and hyperapobetalipoproteinemia have the same disorder of lipoprotein metabolism.

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There is little doubt that the plasma lipoproteins significantly influence the risk of coronary artery disease (CAD). However, this relation is complex with, on the one hand, risk increasing as low-density lipoprotein (LDL) levels increase, but on the other, decreasing as high-density lipoprotein (HDL) levels increase.[1,2] To this long-standing epidemiologic network of evidence can now be added the recent demonstration that appropriate dietary and pharmacologic therapy significantly decreases the incidence of CAD death rate and myocardial infarction (MI) in patients with type II hyperlipoproteinemia.[3]

Even though most patients with premature CAD have LDL cholesterol levels within the normal range, this does not mean their LDL is always normal. LDL are complex particles with the principal lipid, cholesterol ester, sequestered in a core surrounded by a surface layer made up of cholesterol, phospholipid and the major protein in LDL—apoB.[4] Until recently, the plasma concentration of LDL was usually expressed only by its cholesterol content. But this practice provides incomplete information. For example, we observed that although most patients with premature CAD have normal LDL cholesterol levels, many have elevated LDL apoB values—a combination we called hyperapobetalipoproteinemia.[5] And indeed, several studies indicate that apoprotein levels do identify patients at high risk of CAD more clearly than do lipid levels.[5–15]

Although lipoprotein cholesterol levels clearly modulate risk for individuals another body of evidence points to the aggregation of CAD within families, and this phenomenon apparently cannot be completely explained by the factors known to affect coronary risk.[16–18] Therefore the purpose of this study was to examine the plasma lipids, lipoprotein lipids, and plasma LDL apoB levels in children of families where 1 parent had both premature CAD and hyperapobetalipoproteinemia. These data were then compared with those from 3 unrelated control groups of children and young adults to determine whether familial aggregation

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TABLE I Plasma Total Lipid, Lipoprotein Cholesterol and Plasma Low-Density Lipoprotein ApoB Levels in Offspring of Patients with Hyperapobetalipoproteinemia and Myocardial Infarction and Unrelated Control Groups

	MI Offspring	C 1+2+3	C1	C2	C3
TC (mg/dl)	174 ± 34†	164 ± 27	172 ± 19	150 ± 31	164 ± 29
Tg (mg/dl)	103 ± 58‡	81 ± 32	83 ± 21	87 ± 44	78 ± 32
LDL C (mg/dl)	103 ± 33*	93 ± 27		92 ± 30	93 ± 27
HDL C (mg/dl)	51 ± 13	52 ± 11		40 ± 8	56 ± 9
LDL apoB (mg/dl)	110 ± 36‡	82 ± 18	80 ± 16	73 ± 24	87 ± 14

* $p < 0.05$; † $p < 0.01$; ‡ $p < 0.0001$.
C1, C2, C3 = control groups 1, 2 and 3; HDL C = high-density lipoprotein cholesterol; LDL apoB = low-density lipoprotein apoB; LDL C = LDL cholesterol; MI offspring = children of affected parents; TC = total plasma cholesterol; Tg = total plasma triglyceride.

of hyperapobetalipoproteinemia is present within the offspring of the index cases.

Methods

Study groups: *Children of affected parents:* The children of affected parents (MI offspring) consisted of children in 24 families in which 1 parent had both a documented MI before the age of 55 years and hyperapobetalipoproteinemia. All index parents but one were male. Their average age was 48 ± 8 years (mean ± standard deviation) and all had hyperapobetalipoproteinemia, that is, the plasma LDL apoB level was at least 120 mg/dl and the LDL cholesterol was at least 200 mg/dl. Fasting plasma samples were obtained from 66 children and young adults in these families, representing 84% of the total number of offspring. The average age of those studied was 17 ± 7 years (range 3 to 31). Thirty-one were male and 35 were female subjects.

Control groups: Three unrelated groups of children and young adults were studied. Group C1 consisted of 62 healthy Montreal children from whom plasma samples were obtained at a routine health examination. Their average age was 11.6 ± 8 years (range 4 to 23). Twenty-eight subjects were male and 34 were female. Group C2 consisted of 40 children in grade 10, all males, sampled as part of a larger project to determine community lipid and lipoprotein values. These children live in a small Pennsylvania city and their age was 16 ± 1 years. Group C3 consisted of 105 medical students at The Johns Hopkins University with an average age of 20.8 ± 1.5 years (range 19 to 30 years). Ninety subjects were male and 15 were female.

Laboratory methods: After a fast of 12 hours, blood samples were obtained in tubes containing EDTA (1 mg/ml) and plasma then separated by centrifugation. Plasma total cholesterol and triglyceride were measured enzymatically. HDL cholesterol was determined in the supernatant after precipitation of the apoB containing lipoproteins by heparin and manganese chloride.[19] In the MI offspring, LDL was isolated by preparative ultracentrifugation between density 1.006 to 1.063 g/ml.[5] That is, the density <1.006 lipoproteins were first removed and then LDL isolated after a second ultracentrifugation at density 1.063 g/ml. In groups C2 and C3, LDL cholesterol was determined using the methods of the Lipid Research Clinic.[19] Plasma LDL apoB was measured in all groups by radial immunodiffusion.[20] In groups MI, C1 and C3, this was done at the Royal Victoria Hospital laboratory using a 1.5% agarose gel read at 18 hours. In groups C2, radial immunodiffusion was performed at The Johns Hopkins laboratory using a 2% agarose gel read at 72 hours. The measurements in the 2 techniques agree closely ($r = 0.96$).

Normal limits for plasma lipids and lipoprotein lipids were based on the Lipid Research Clinics Prevalence Study.[21] The upper limit, based on our previous studies in adults for plasma LDL apoB, was fixed at 120 mg/dl.

Groups were compared initially with t tests with the data then adjusted for age and sex effects by analysis of covariance. Multiple logistic regression was then employed to search for the most informative parameters.[22]

Results

The means and standard deviations for all measurements are summarized in Table I. Each of the 3 control groups is listed separately and these values are averaged to provide an overall mean for the control group. The values for the MI offspring were then compared with those of the overall control group.

First, a univariate comparison was carried out using an unpaired t test. This revealed that plasma total cholesterol, total triglyceride, and plasma LDL apoB levels were all significantly higher in the MI offspring than in the control group. By contrast, the mean HDL cholesterol levels did not differ significantly between these 2 groups. The influence of age and sex was next considered, and these factors were determined in the control group with all data then adjusted by analysis of covariance. This altered the absolute differences between the groups only slightly and the differences noted above remained just as significant.

Multivariate analysis was then undertaken by multiple logistic regression: plasma LDL apoB was the parameter selected first as significant with LDL cholesterol identified next. Addition of the 3 other lipid variables did not substantially improve the fit. Thus, multivariate analysis identified plasma LDL apoB and then LDL cholesterol as the measurements that permit best separation of the MI offspring and control group.

These differences can also be appreciated by comparing the difference between the means of the control and MI offspring of a given measurement as a fraction of the control standard deviation. Thus, the mean for plasma total cholesterol in the MI offspring differs from the mean in the control offspring by much less than 1 standard deviation. This is also the case for LDL cholesterol, while the difference is only a bit larger (0.62) for triglyceride. By contrast, there is a much larger difference between the 2 groups for plasma LDL apoB. In this instance, the difference is 1.5 times the control standard deviation. So that the magnitude of difference

between the 2 groups for LDL cholesterol and LDL apoB can readily be seen, histograms for these 2 variables are shown in Figure 1.

Table II lists the persons in the MI offspring with elevated plasma LDL apoB. The youngest was 4 years old, a boy, and the oldest was 33 years old, a woman. Of these 22, 4 had LDL cholesterol values above the 95th percentile for age and sex and, thus, would be recognized as abnormal; 2 others were just at this limit. One young adult with an increased LDL cholesterol also had an elevated plasma triglyceride level, whereas 3 others had increased plasma triglyceride, but normal LDL cholesterol levels; 2 had plasma triglyceride levels at the 95th percentile. Thus, only 7 of the 22 had clearly abnormal lipid levels; the rest, were it not for plasma LDL apoB, would have been conventionally characterized as normal.

Discussion

This study examines children and young adults in families where a parent had both suffered a premature MI and had hyperapobetalipoproteinemia. Many of the offspring also have hyperapobetalipoproteinemia; that is, they had elevated LDL apoB levels in the face of a normal LDL cholesterol. Only a few had elevated LDL cholesterol or plasma triglyceride levels, and thus measurement of plasma and lipoprotein lipid variables did not clearly identify many persons within the MI offspring as being different from the 3 control groups of children and young adults. By contrast, measurement of plasma LDL apoB differentiated the primary study group from the 3 control groups, also identified the few children with elevated LDL cholesterol, but more importantly, identified the larger number with hyperapobetalipoproteinemia.

Familial aggregation of CAD has been demonstrated repeatedly. The Western Collaborative Study[23] revealed a significant link between the history of CAD and parental history of CAD; while Slack and Evans showed a several-fold excess mortality in the relatives of index cases with CAD compared to the normal population.[24]

Nevertheless, the connection between familial aggregation of CAD and familial aggregation of lipids is clearly imperfect.[16–18] More recently, however, the relation of apoproteins to risk of CAD has been examined. Although most patients with premature CAD are normocholesterolemic,[5,25] there is now considerable agreement that, on average, apoB levels are higher and $apoA_1$ (the major apoprotein in HDL) levels lower in patients with CAD than in those without disease.[5–15] Nevertheless, all such studies on the relation of apoprotein levels to atherosclerosis have been cross-sectional since the presence or absence of disease was related to concurrent lipid and apoprotein levels. Therefore, the apoprotein abnormalities observed may have been simply a consequence, rather than a precursor, of vascular disease. The present data indicates that hyperapobetalipoproteinemia is expressed in families with premature CAD long before the usual age when clinical symptoms begin, and therefore contributes strongly to the hypothesis that this metabolic disorder may well be important in the pathogenesis of CAD. Our hypothesis is further strengthened by the results of Kukita et al,[26] who reported that apoB levels in first-degree adult relatives of patients with angiographically diagnosed CAD were significantly higher than those in age- and sex-matched control subjects.[26]

Even though a child as young as 4 years was affected, most of those with hyperapobetalipoproteinemia were beyond puberty, and thus it is possible that this disorder is not fully expressed until young adulthood. In the present study, the control groups were chosen not only to bracket the age range in the primary study group, but

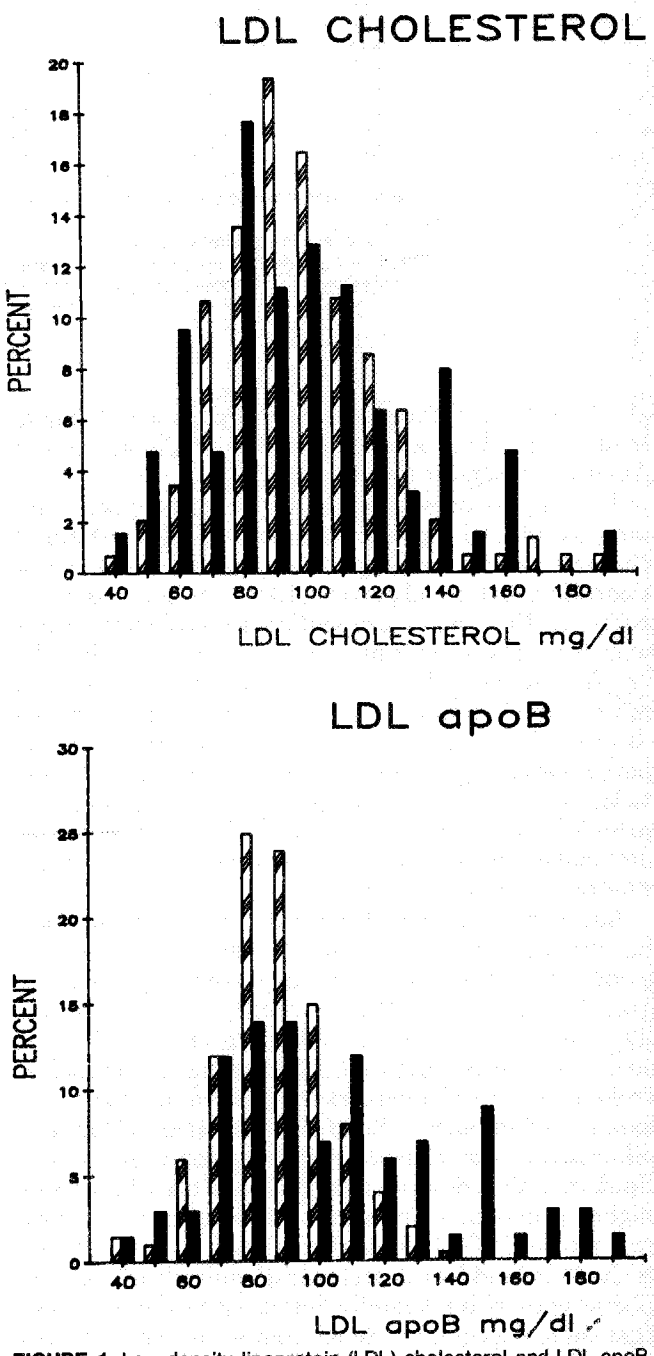

FIGURE 1. Low-density lipoprotein (LDL) cholesterol and LDL apoB levels for both the control group (**hatched bars**) and the children of affected parents (**solid bars**).

TABLE II Children of Affected Parents with Elevated Plasma Low-Density Lipoprotein ApoB Levels

Age (yr) & Sex	Tg	TC	LDL apoB	LDL C	HDL C
4M	42	180	137	130	40
14F	184 (131)	216	155	120	54
22M	198 (165)	242	129	148 (147)	61
25F	181 (172)	189	153	132	29
23F	157	224	205	165 (159)	43
19F	145	210	195	141	31
22M	183	206	130	148 (147)	32
21F	98	267	175	215 (164)	32
17F	94	210	121	162 (137)	26
8M	101 (101)	182	124	120	40
12F	86	217	139	159 (136)	46
11F	25	196	155	113	68
11F	60	200	128	113	75
25F	133	221	190	148	43
23F	152	217	160	123	64
19F	97	177	142	116	45
25M	95	137	131	77	40
20F	169 (165)	180	154	103	47
26M	173	190	138	106	54
27M	179	234	153	169 (165)	32
26F	402 (172)	240	170	104	56
27F	139	197	154	126	43

Figures in parentheses are the 95th percentile of age and sex values based on the Lipid Research Clinics Survey.

All values are in milliliters per deciliter.

HDL C = high-density lipoprotein cholesterol; LDL apoB = plasma low-density (LDL) apoB; LDL C = LDL cholesterol; LP = lipoprotein phenotype; TC = total plasma cholesterol; Tg = plasma triglyceride.

also to be from different geographic sites so as to make them more likely a representative of Canadian and American norms at these ages.

The physiochemical basis for the disproportionate increase in LDL apoB compared to LDL cholesterol characteristic of hyperapobetalipoproteinemia is now clear. Even in normal persons, LDLs are heterogeneous particles differing in size and cholesterol content but with a constant amount of protein per particle. The larger LDL particles are thus less dense and contain more core cholesterol, but relatively less protein; the converse is the case for smaller LDL particles. In hyperapobetalipoproteinemia, the number of LDL particles is above normal, but the cholesterol level is not proportionately elevated because most of the particles are smaller and denser, depleted in cholesterol ester and relatively enriched in protein.[27]

Although in no way established, it is possible that the familial aggregation of hyperapobetalipoproteinemia evident in the present study may to an important degree, be genetic, and this, obviously, is an important hypothesis to pursue. Of the lipoprotein disorders associated with premature CAD, familial hypercholestrolemia is best characterized, but accounts for only a small portion, about 5%, of the premature CAD seen clinically. Another disorder, familial combined hyperlipidemia, first described in 1973, has been found in a higher proportion, from 10 to 20%, of patients with premature CAD.[28] Familial combined hyperlipidemia is characterized by the presence of multiple lipoprotein phenotypes within a family, but it has now been shown that there is a common denominator linking these various phenotypes—an elevated total plasma apoB.[29] Thus, there may be considerable, although not complete, homology between hyperapobetalipoproteinemia and familial combined hyperlipidemia.[30]

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